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Microwave-assisted synthesis of unnatural amino acids

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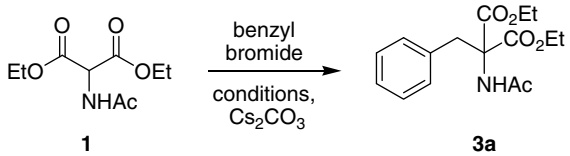
ABSTRACT

Microwave irradiation has been proven to be a useful tool in the rapid assembly of racemic unnatural amino acids in only two steps. Additional benefits of this methodology are the commercial availability of the inexpensive starting materials and the high yields and high purities of the final amino acid products.

Microwave irradiation has become a very useful tool in synthetic organic chemistry, and has been employed in a variety of reactions.[1] Microwave-assisted synthesis has been found to increase both reaction rates and yields via more efficient heating compared to standard thermal conduction. Moreover, in certain cases specific microwave effects have been discussed which facilitate reactions that can not be conducted under purely thermal heating.[2] In order to further probe the utility of microwave irradiation in organic synthesis we investigated its utility in the preparation of amino acids and unnatural amino acid analogs. The synthesis of unnatural amino acids is especially interesting due to technological advances in their incorporation into proteins both in vivo and in vitro.[3] Since unnatural amino acids can have a repertoire of functional groups that vastly extends beyond the common set of natural amino acids, they can be used as probes to obtain a better understanding of biological processes. While several routes to amino acid synthesis currently exist, microwave irradiation has rarely been employed.[4] To the best of our knowledge, only one synthetic route to unnatural amino acids using microwave irradiation has been attempted via the Michael addition of methyl *N*-(diphenylmethylene)-2,3-didehydroalaninate and nitroalkanes.[5] Herein we explore a more general microwave-assisted alkylation approach to afford a wide variety of unnatural amino acid derivatives in a facile fashion.

Initial investigations commenced with the optimization of the alkylation of diethyl acetamidomalonate (**1**) with benzyl bromide (**2a**) in the presence of base to yield the diester **3a** (Table 1). Several bases were examined including NaH, NaOEt, K_2CO_3 and Cs_2CO_3. Ultimately Cs_2CO_3 was found to be optimal as it afforded high yields with a minimum number of side products compared to the other bases. We next investigated the role of the solvent and the microwave power on the alkylation reaction (Table 1). Based on our previous experience in microwave-mediated reactions,[6] tolu-

Table 1
Optimization of the microwave-assisted alkylation reaction



1 **3a**

Entry	Conditions	MW power (W)/temperature (°C)	Yield **3a**
1	PhMe, 10 min	300/25–120[a]	31%
2	DMF, 10 min	0–200/170[b]	45%
3	THF, 10 min	300/25–85[a]	59%
4	THF, 10 min	200/25–80[a]	47%
5	**ACN, 10 min**	**0–300/130[b]**	**82%**
6	ACN, 10 min	0/130	43%

[a] Fixed microwave power input and variable temperature.
[b] Fixed temperature and variable microwave power input.

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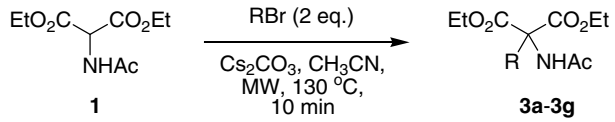
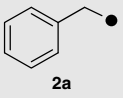
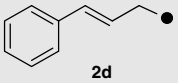
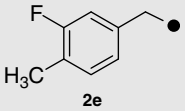
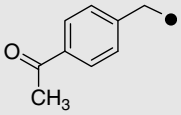
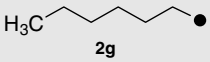
ene was employed as a microwave transparent solvent, thus enabling a high level of microwave power input to the substrates. However, even at 300W of microwave irradiation overall conversion was low, presumably due to the poor solubility of Cs_2CO_3. When the alkylation was conducted in DMF, a polar, strongly microwave absorbing solvent, a temperature of 170 °C was reached very quickly; however, the yield of **3a** was still relatively low at 45%. Conducting reactions in microwave absorbing solvents limits the ability to use high microwave power inputs due to the rapid heating of the solvents beyond their boiling point, leading to a substantial pressure formation in the sealed reaction vessel. Based on these observations, we next performed the reaction in microwave transparent THF, resulting in decreased temperatures but also significant amounts of the starting material **1**. However, these conditions afforded slightly higher yields of **3a** at high microwave powers: 47% yield at 200W and a 59% yield at 300W. We then conducted the reaction in acetonitrile, which is a common solvent employed in similar alkylations.[7] Acetonitrile was found to be the optimal solvent under standard microwave irradiation at ~130 °C for 10 min affording **3a** in 82% yield. When this reaction was conducted thermally at 130 °C, under otherwise identical conditions, 43% of **3a** was obtained.

With optimized conditions in hand (Cs_2CO_3, CH_3CN, 130 °C, microwave irradiation), the reaction scope was investigated with several bromides (**2a–2g**) to yield the alkylated products **3a–3g** (Table 2). Overall the amino acid precursors **3a–3g** could typically be prepared in moderate to high yields within a short 10 min reaction time. The methodology tolerates a wide range of functionalities including alkyl (**2g**), aryl (**2a**, **2d**, and **2e–2f**), alkene (**2b** and **2d**), alkyne (**2c**), fluorine (**2e**), and ketone (**2f**) functional groups. However, aliphatic chains (**2g**) lead to reduced yields despite further optimization attempts, presumably due to their intrinsic lower reactivity and their propensity to undergo eliminations. In order to increase the yield for certain bromides (**2b**, **2e**, and **2g**), sodium iodide (20 mol%) was added to the reaction mixture to facilitate substitution via an in situ leaving group exchange, increasing the yields by 10–15%.

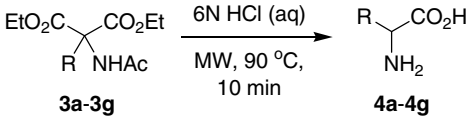
Table 2
Preparation of unnatural amino acid precursors **3**



Entry	R Group	Product	Yield
1	 2a	**3a**	82%
2	 2b	**3b**	78%[a]
3	 2c	**3c**	68%
4	 2d	**3d**	76%
5	 2e	**3e**	73%[a]
6	 2f	**q**	92%
7	 2g	**3g**	33%[a]

[a] Sodium iodide (20 mol%) was added.

Table 3
Conversion of precursor **3** to amino acid **4**



Entry	Diester	Product	Yield (%)
1	**3a**	**4a**	98
2	**3b**	**4b**	88
3	**3c**	**4c**	90
4	**3d**	**4d**	94
5	**3e**	**4e**	86
6	**3f**	**4f**	96
7	**3g**	**4g**	92

After generating a small array of the amino acid precursors **3a–3g**, a global deprotection of the carboxy and the amino protecting groups followed by an instant decarboxylation was performed to yield the amino acids **4a–4g** in a single step. Typically, this reaction is conducted in refluxing aqueous 6 N HCl for approximately 12 h. However, we discovered that this transformation also benefits from microwave irradiation. After just 10 min of microwave irradiation of **3a–3g** in 6 N HCl, the amino acids **4a–4g** were obtained in excellent yields of 85–99% and purities of >95%, as determined by ^{1}H NMR (Table 3). The analytical data of **4a–4g** was in agreement with literature reports.[8] Thermal reactions mimicking the microwave reaction conditions afforded minimal product conversion <20%, and significant amounts of the starting materials **3a–3g** were retained. Overall, the preparation of racemic unnatural amino acids was achieved in a combined 20 min of reaction time with a single chromatography purification between reactions.[9]

In conclusion, a new microwave-assisted two-step methodology to rapidly prepare racemic unnatural amino acids in high yield and high purity was developed. Due to the wide range of functional groups tolerated via this approach, several important unnatural amino acids were attained. Upon incorporation into proteins and peptides these amino acids can potentially be employed in bioconjugation reactions (**4c** and **4f**),[10] as NMR labels (**4e**),[11] and in photochemical transformations (**4b**).[12] Due to the high convergence of this approach and the wide range of inexpensive commercially available bromides, the methodology will find widespread application in the rapid assembly of unnatural amino acids and derivatives thereof.

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9. Diethyl acetamidomalonate (50 mg, 0.23 mmol), Cs_2CO_3 (150 mg, 0.46 mmol, 2 equiv **2** (0.46 mmol), and acetonitrile (3 mL) were added to a flame dried microwave vial. The vial was place in a CEM Discover microwave reactor and irradiated in standard mode (130 °C) for 10 min. The reaction mixture was cooled to room temperature, filtered to remove Cs_2CO_3, and concentrated under reduced pressure. Purification via column chromatography (Hexane/EtOAc) yielded pure alkylation products **3a**–**3g**. These compounds were dissolved in 6 N HCl (aq.) and irradiated for 10 min in a CEM Discover microwave reactor (standard mode, 90 °C). Removal of the volatiles under reduced pressure yielded pure **4a**–**4g** without further purification.
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